

# Protein Detection in Gels and on Membranes

## Novel Time-resolved Fluorescence Approach for Planar Surfaces

Protein analysis using one- and two-dimensional (1D- and 2D-) gels is a central part of pharmaceutical and clinical research. Currently the most sensitive protein detection relies on fluorescence staining, which is limited due to high background fluorescence signals of the gel matrix or membranes where the proteins can be transferred to (electroblotting). Time-resolved fluorescence (TRF) bears the potential to detect the protein signal after decline of the background thus obtaining higher signal to noise ratios. Here we describe a novel detection platform for measuring TRF signals in gels and on membranes.



### Introduction

Current proteomic strategies play a central role for pharmaceutical and clinical research. For most diseases, proteins are seen as the central key to their understanding and though for the development of diagnostic and prognostic techniques as well as targets for specific treatments.

Some of the best established and most commonly applied methods in proteomics include SDS-PAGE and 2D-gel electrophoresis with the capability to separate proteins in complex mixtures. The separated proteins can be either directly visualised in the gel or transferred first to solid supports like PVDF or nitrocellulose membranes. For visualisation, fluorescence dyes are commonly used but their sensitivity is limited due to high background fluorescence signals of the above mentioned gels or membranes. Time resolved fluorescence (TRF) is capable to overcome these limitations.

The TRF technology is based on beneficial fluorescence properties of lanthanide chelate labels like Europium(III)- or Terbium(III)-complexes. The fluorescence lifetime of the lanthanide labels is several orders of magnitude longer than for the background. Recording the lanthanide fluorescence after the background has decayed results in a much better signal to

noise ratio. This method is already used for assays in liquid phase, but has not been applied to scan planar surfaces like gels or membranes. Here, we demonstrate the use of a TRF dye on the Beckman Coulter Paradigm Detection Platform for protein detection on PVDF membranes and in polyacrylamide gels.

For the TRF measurements, a modified version of the "TRF Detection Cartridge" was used on the platform. Membranes or gels are directly scanned on a compatible gel and membrane tray. The Multimode Analysis Software offers predefined detection methods using timing-parameter protocols optimised for certain TRF measurements.

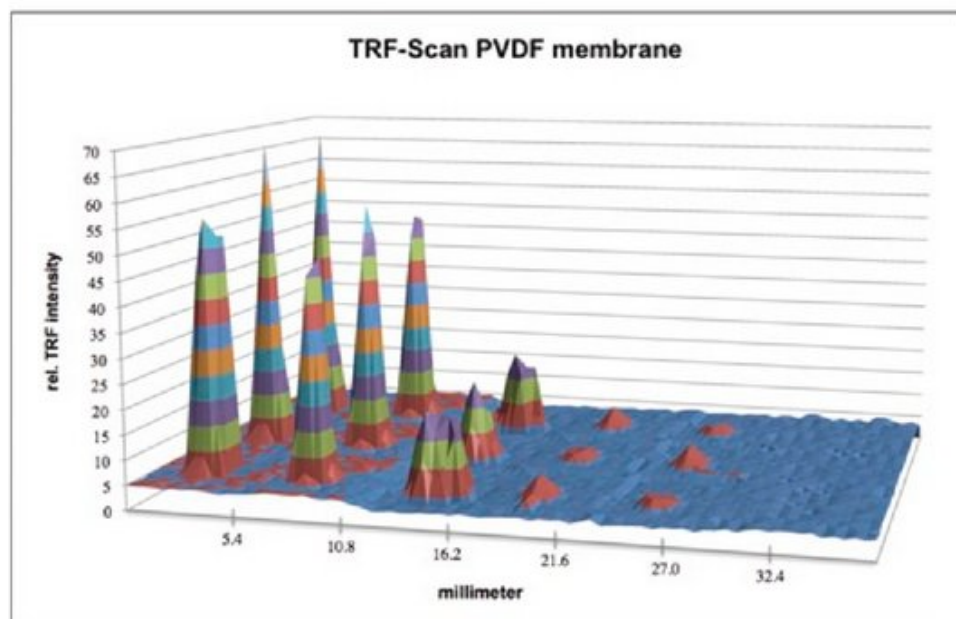


Fig. 1: TRF dye spotted onto PVDF membrane. Y-axis shows relative time-resolved fluorescence intensities. Area between X- and Z-axis represent scanned surface areas of the PVDF membrane. TRF dyes were spotted in triplicates. Highest concentration used equals to 1 pmol of TRF dye.

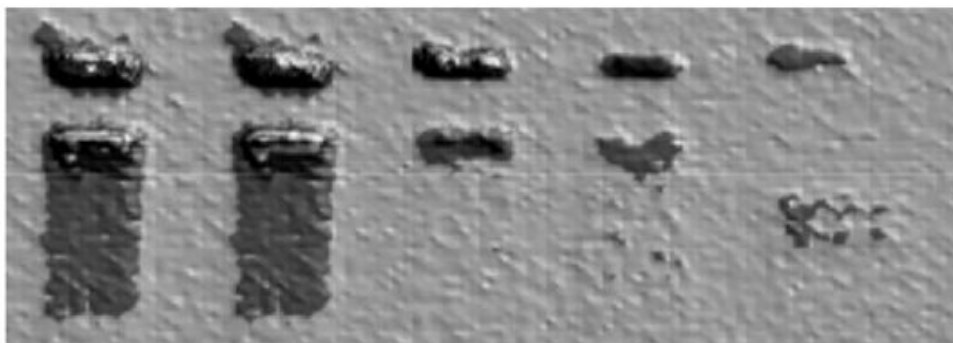


Fig. 2: TRF scan of a polyacrylamide gel showing TRF dye in the upper to lower fmol range. Scan was performed with a custom made TRF Detection Cartridge of the Beckman Coulter Paradigm Detection Platform.

## Results

In a first experiment, a TRF dye was spotted onto a PVDF membrane and detected with a modified version of the "Time Resolved Fluorescence Detection Cartridge" (fig. 1). Spots of decreasing concentrations of the TRF dye were spotted on a PVDF membrane in triplicates. At the highest concentration, 1 pmol of TRF dye was spotted, yielding a signal to background ratio of approximately 13 to 1. Signal linearity could be achieved over several orders of magnitude and the signal intensities were reproducible within a standard deviation of less than 6%.

Furthermore, the detection system is capable of measuring samples in SDS polyacrylamide gels, as shown in figure 2 for a dilutions series from the upper to the lower fmol range. Scan resolution can currently be used down to 0.225 mm.

## Discussion

The data presented here demonstrate that this instrument is ideal to combine diverse spectrophotometric measurement capabilities using 6 well- up to 3456 well-plates with a TRF surface area scan mode for gels and membranes. Both sensitivity and reproducibility of the scans

were excellent and meet the demands of a fast growing market for sensitive protein detection and quantification. Integration of recording fluorescence intensity, fluorescence polarisation, luminescence, absorption and time resolved fluorescence in both the microwell-plate format and on planar surfaces in a single instrument is unique.

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## PRODUCTS

## Fully Integrated

Intelligent Imaging Innovations' (3i) Marianas SDC is a fully integrated spinning disk confocal workstation based on motorised Zeiss microscopes. It can be combined with a variety of microscopy applications, such as FRAP, Photoactivation, FLIM and TIRF sharing the same hardware (lasers, cameras). This allows for an easy and cost-effective upgrade from basic to advanced systems. Prominent features are e.g.: Yokogawa CSU-X1 spinning disk confocal head, Laserstack Launch with up to five selectable laser lines, latest EMCCD camera technology, and an option for both confocal and widefield (deconvolution) image paths. Also optional: point-scanning and whole array FRAP/photoactivation, frequency-domain FLIM module, high-speed 4D and simultaneous Dual-Camera detection, and a TIRF module.



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## New Real-Time Microarray Platform

HybLive, the recently developed open real-time microarray platform from Genewave integrates hybridisation, washing, and detection functionalities within a single bench-top instrument, thus facilitating full microarray-based assay automation. In addition, and in contrast to standard tools, HybLive system makes it possible to monitor target molecule hybridisation and melting in real time, directly on the chip. This innovation opens the way to many applications that were inaccessible until now, such as on-chip probe verification and validation or the definition of optimal hybridisation conditions over a single experiment, melting curve measurement or the study of on-chip intermolecular interactions, SNP detection, etc. The system is compatible with standard format microarray slides and offers the possibility of using up to four detection channels (colours). HybLive platform is the ideal tool for DNA or protein microarray assay development, and may also be routinely used in basic and clinical research.



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